

## ORIGINAL ARTICLE

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## A community outbreak of Legionnaires' disease: evidence of a cooling tower as the source

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### ABSTRACT

A community outbreak of *Legionella* pneumonia in the district of Cerdanyola, Mataró (Catalonia, Spain) was investigated in an epidemiological, environmental and molecular study. Each patient was interviewed to ascertain personal risk-factors and the clinical and epidemiological data. Isolates of *Legionella* from patients and water samples were subtyped by pulsed-field gel electrophoresis. Between 7 August and 25 August 2002, 113 cases of *Legionella* pneumonia fulfilling the outbreak case definition criteria were reported, with 84 (74%) cases being located within a 500-m radius of the suspected cooling tower source. In this area, the relative risk of being infected was 54.6 (95% CI 25.3–118.1) compared with individuals living far from the cooling tower. Considering the population residing in the Cerdanyola district (28 256 inhabitants) as a reference population, the attack rate for the outbreak was 399.9 cases/100 000 inhabitants, and the case fatality rate was 1.8%. A single DNA subtype was observed among the ten clinical isolates, and one of the subtypes from the cooling tower matched exactly with the clinical subtype. Nine days after closing the cooling tower, new cases of pneumonia caused by *Legionella* ceased to appear. The epidemiological features of the outbreak, and the microbiological and molecular investigations, implicated the cooling tower as the source of infection.

**Keywords** Cooling towers, epidemiology, *Legionella pneumophila*, outbreak, pneumonia, risk-factors

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### INTRODUCTION

*Legionella* is considered to be responsible for 2–13% of cases of community-acquired pneumonia requiring hospitalisation [1–3]. In a Catalan study, *Legionella pneumophila* accounted for 12.5% of cases of community-acquired pneumonia, with *Streptococcus pneumoniae* being the most frequent cause [4]. However, *Legionella* was the second most frequent causative pathogen in patients with severe pneumonia admitted to the intensive care unit [4,5]. *Legionella* transmission has been associated mainly with the inhalation of aerosols containing the microorganism. However, micro-

aspiration has also been implicated, especially in hospitalised patients [6].

Several environmental sources have been associated with *Legionella* outbreaks, including whirlpool spas [7,8], ornamental fountains [9,10] and the water distribution systems of homes [11], hotels [12] and ships [13]. Colonisation of cooling towers and evaporative condensers by *Legionella*, with the subsequent production of aerosols, has been identified as one of the major sources of community outbreaks of *Legionella* infection [14–17]. However, the evidence of a causal association with a cooling tower has been weak for several community clusters of cases, and molecular studies have been lacking. Consequently, the importance of cooling towers as a source of infection has been questioned [18], although the investigation of an outbreak is difficult because of the frequent delay in

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reporting cases and the difficulty in locating possible sources. Moreover, the condition of the water when the samples are taken is often not the same as at the time of exposure. The present study reports the features of a community outbreak of pneumonia caused by *Legionella* which affected 113 individuals in Mataró, a town of 100 000 inhabitants in Catalonia, Spain, during August 2002. Epidemiological and molecular data that allowed a cooling tower to be identified as the direct cause of the outbreak are presented.

## MATERIALS AND METHODS

### Case definition

A case was defined as an individual who presented with clinical symptoms of pneumonia between 15 July and 25 August 2002, who had resided in or visited the Cerdanyola district of Mataró during the 10-day period before the onset of symptoms, with at least one of the following results: (1) isolation of *Legionella* from respiratory secretions; (2) a positive direct immunofluorescence test for *Legionella*; (3) detection of *L. pneumophila* serogroup (sg) 1 antigen in urine; or (4) a four-fold increase in antibody titres to  $1 \geq 128$  paired serum samples.

### Epidemiological investigation

Face-to-face interviews were conducted by trained researchers with each definitive case to ascertain personal risk-factors and the relevant clinical and epidemiological data. Information concerning each patient's history in the 10-day period before the onset of symptoms was collected, with particular attention to possible exposure to *Legionella* in the interior of buildings, in the open air, or by consumption of water.

### Environmental investigation

Two study areas were defined: Area 1 comprised the entire district of Cerdanyola and adjacent districts within a radius of 1500 m; Area 2 comprised the remainder of the municipality of Mataró.

Possible environmental sources of infection in the two study areas were selected on the basis of data provided by the Town Council. The order of priority for the investigation and collection of samples was established: first, cooling towers and evaporative condensers located in Area 1; second, ornamental fountains located within 500 m of the cases, and the sprinklers and street cleaning facilities of the district; and third, the water supply, cooling towers and evaporative condensers in Area 2. Two methods were used to detect environmental sources not included in the census: first, documentary investigation of companies who had applied for an activity licence from the Town Council; and second, a field investigation of equipment located on the roofs of buildings using binoculars and cameras, followed by a physical investigation of the premises. A visual inspection was also made from a helicopter using thermal imaging to measure heat emissions.

### Microbiological and molecular analyses

The water samples (1 L) were concentrated and decontaminated by acid treatment, followed by inoculation in duplicate on selective GVPC-BCYE (Glycine Vancomycin Polymyxin B Cycloheximide-Buffered Charcoal Yeast Extract Agar; Oxoid, Wesel, Germany). Isolates of *Legionella* spp. were identified by growth on BCYE, and absence of growth on sheep blood agar plates (bioMérieux, Lyon, France), and by Gram's stain. *L. pneumophila* was identified by the Monofluo IFA test kit (Genetic Systems Corp., Redmond, WA, USA). *L. pneumophila* isolates were differentiated as sg 1 or sgs 2–14 by immunoagglutination serotyping with the MicroScreen *Legionella* Latex Kit (Microkit Iberica, Madrid, Spain). In total, 25 isolates of *L. pneumophila* sg 1 from ten patients (ten isolates) and two towers (seven isolates from one cooling tower and eight from a second tower) were analysed by pulsed-field gel electrophoresis (PFGE) as described previously [19].

### Statistical analysis

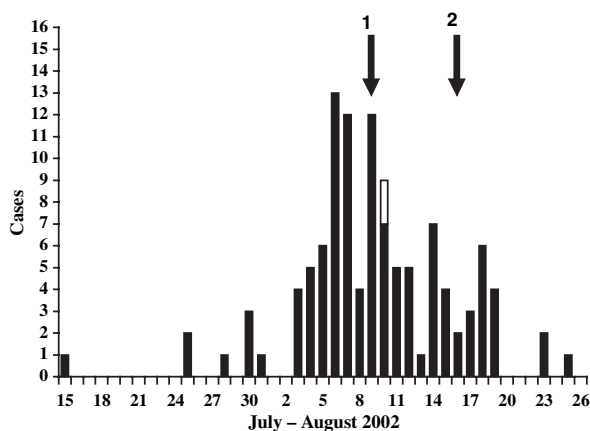
The attack rate of the outbreak with respect to the resident population of the Cerdanyola district of Mataró, as well as the relative risk of becoming ill with respect to the distance from the source of the outbreak of the residence of each patient (< 500 m; 500–1000 m; > 1000 m), were calculated. The chi-square test was used for statistical analysis of qualitative variables, and the Student's *t*-test was used for quantitative variables. The level of statistical significance was *p* 0.05.

## RESULTS

### Case finding and descriptive epidemiology

On 7 August 2002, two definitive cases of *Legionella* pneumonia in residents of the municipality of Mataró were reported to the Department of Health of the Generalitat of Catalonia. On 9 August 2002, an active case-finding investigation was initiated, with 113 definitive cases of *Legionella* pneumonia identified in the period until 25 August 2002. The onset of symptoms in the first case was on 15 July, and in the last case on 25 August. Between 7 August and 25 August, 32 cases of community-acquired pneumonia that did not fulfil the diagnosis criteria for *Legionella* infection were also identified (Fig. 1).

Seventy-four percent of the cases resided within a 500-m radius of the suspected source (see below), representing an incidence rate of  $509.7/10^5$  per month. An additional 24% of cases resided within a radius of 500–1000 m, with an incidence rate of  $110.7/10^5$ , with 6% of cases, an incidence rate of  $9.3/10^5$ , residing in the area (although all cases had carried out some activity within the 1000-m radius). An evaluation of the



**Fig. 1.** Epidemic curve showing the date of onset of symptoms. Arrow 1, day of detection of outbreak (9 August); arrow 2, day of detection of the likely source of the outbreak (16 August). The white bar on day 10 indicates the two patients who died during the outbreak.

risk of becoming infected according to residence in each zone, taking the incidence in the external area as the reference, showed that residents living within 500 m of the suspected source had a relative risk of 54.6 (95% CI 25.3–118.1), and that residents living within 500–1000 m had a relative risk of 11.9 (95% CI 5.1–27.8).

Considering the residents of the Cerdanyola district (28 256 inhabitants) as a reference population, the attack rate of the outbreak was 399.9 cases/100 000 inhabitants.

### Patient characteristics

The median age of the 113 patients was 59.4 years (SD  $\pm$  16.8 years), with 73 (64.6%) males (mean age 55.5 years; SD  $\pm$  15.0 years) and 40 (35.4%) females (mean age 66.8 years; SD  $\pm$  17.5 years;  $p$  0.001). The most frequent personal risk-factor for acquisition of the disease was a history of smoking tobacco (46/113). Other risk-factors were diabetes (28/113), chronic bronchitis (12/113), and cancer (7/113). No personal risk-factors for the disease were found in 31.9% (36/113) of patients, a figure which fell to 10.6% (12/113) when age  $>$ 60 years was included as a risk-factor. Table 1 shows the patient characteristics, grouped according to whether the onset of symptoms was before or after 9 August, which was the date the outbreak was recognised and the information was made public. Of the cases, 73.5% (83/113) were hospitalised, with a median stay of 6 days.

**Table 1.** Patient characteristics, grouped according to whether symptoms began before or after the day of recognising and informing the public about the outbreak

Patient characteristics	Total	Before	After	p
Number of cases	113	52	61	
Mean age, years	59.4 ( $\pm$ 16.8)	61.6 ( $\pm$ 16.7)	57.7 ( $\pm$ 16.8)	0.220
Males	64.6%	51.9%	75.4%	0.009
Smokers	40.7%	32.7%	47.5%	0.109
Chronic bronchitis	10.6%	9.6%	11.5%	0.749
Diabetes	24.8%	28.8%	22.8%	0.474
Case fatality rate	1.8%	0%	3.3%	0.499
Hospitalisation	73.5%	86.5%	62.3%	0.004
Mean interval (days) between onset of symptoms and hospitalisation	5.2 ( $\pm$ 3.4)	6.3 ( $\pm$ 3.9)	4.3 ( $\pm$ 2.7)	0.002

Two patients died, giving a case fatality rate of 1.8% (2/113).

### Assessment of exposure

An assessment of exposure could be made for 104 (92%) of the 113 patients. The most frequent factor involved walking in parks and gardens (54/113), with nine different locations being mentioned. Shopping in large commercial centres was a common factor for 27.4% (31/113) of the cases, with six different locations being mentioned. With respect to the water consumed during the 10-day period before the onset of symptoms, 47.8% (43/90) drank tap water, 51.1% (46/90) drank bottled water, and 1.1% (1/90) drank water from other sources (spring water).

### Environmental investigation

An investigation of the possible environmental sources of *Legionella* was initiated on 9 August 2002. Thirteen cooling towers or evaporative condensers were located and studied. Five towers were located inside Area 1 and eight inside Area 2. In addition, four ornamental fountains, five sprinkler heads, three water cisterns for street cleaning, two water company cisterns and the water systems of three private houses were investigated and sampled.

The search for undisclosed towers revealed five towers, one of which, detected on 16 August 2002 (i.e., 8 days after the recognition of the outbreak), was a working cooling tower providing refrigeration for an ice-making factory located in the middle of the Cerdanyola district. The tower was a small (1  $\times$  1  $\times$  2 m) mechanical draft tower, located within the factory walls, but emitting

aerosols directly to the street from a height of c. 2 m. The only maintenance measures involved replacing part of the re-circulating water daily and adding 150 mL of chlorine weekly. On 16 August, following water sampling, the tower was shut down because of its location (the majority of cases clustered around the tower) and its poor standard of maintenance.

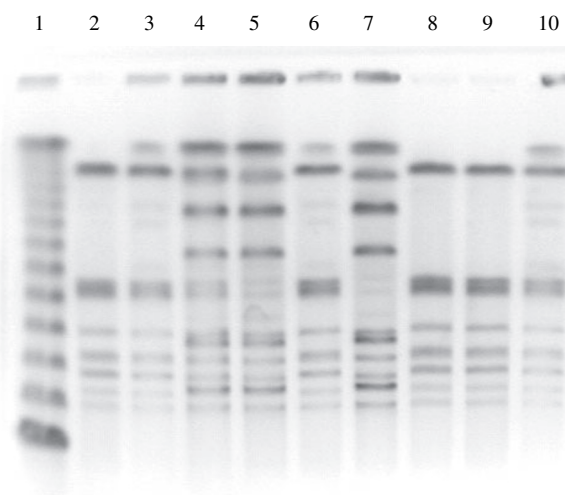
### Microbiological and molecular analyses

Of the 13 samples taken from cooling towers and evaporative condensers, two were positive for *L. pneumophila* sg 1, three were positive for *L. pneumophila* sgs 2–14, and eight were negative. The sample corresponding to the cooling tower of the ice-making factory was positive for *L. pneumophila* sg 1 ( $2 \times 10^5$  CFU/L). The samples from ornamental fountains, water cisterns, the public water system, and systems from certain private dwellings, were all negative for *Legionella* spp. Of the five sprinklers tested, one was positive for *L. pneumophila* sgs 2–14 ( $10^2$  CFU/L).

Table 2 shows the microbiological data for each facility tested. A single DNA subtype was observed among the ten clinical isolates (subtype A). The isolates of *L. pneumophila* sg 1 from the two positive cooling towers exhibited four DNA subtypes, two in each tower (subtypes A and B in the cooling tower of the ice-making factory, and subtypes C and D in a second cooling tower). Subtype A from the cooling tower in the ice-making factory was indistinguishable from the clinical subtypes (Fig. 2).

**Table 2.** Summary of microbiological results according to type of facility

Type of facility	n	Results of analyses			
		Positive for <i>Legionella pneumophila</i> serogroup 1 (> $10^3$ CFU/L)	Positive for <i>Legionella pneumophila</i> other serogroups	Negative for <i>Legionella pneumophila</i>	Not available
Cooling towers or evaporative condensers					
Area 1, < 1500 m	5	1	1	3	0
Area 2, ≥ 1500 m	8	1	2	3	2
Ornamental fountains	4	0	0	4	0
Sprinklers	5	0	1	4	0
Street cleaning systems	3	0	0	3	0
Public water supply	2	0	0	2	0
Private dwellings	3	0	0	3	0
Total facilities sampled	30	2	4	22	2



**Fig. 2.** DNA subtypes identified by pulsed-field gel electrophoresis among isolates of *Legionella pneumophila* sg 1. Lanes: 1, lambda size marker; 2 and 3, clinical isolates (subtype A); 4–10, cooling tower isolates (subtypes A and B).

### DISCUSSION

The community outbreak of Legionnaires' disease described in the present study is one of the largest reported in terms of the number of cases and the attack rate. The outbreak investigation identified a specific cooling tower as the source of the outbreak, and suggested that aerosolisation was the most probable transmission mechanism. Cooling towers have been implicated in many outbreaks of Legionnaires' disease [14–17], but the evidence has rarely been as conclusive as in the present study. At the beginning of the epidemiological investigation, the descriptive data did not suggest a specific source. Therefore, all possible sources were investigated, including the municipal water supply and the dwellings of several infected patients. Although the dimensions of the outbreak meant that these were unlikely sources, the possibility of such sources exists for sporadic cases or small outbreaks [20].

The high attack rate, the small area in which the cases appeared, and the elimination of the less likely sources, made it necessary to search for facilities capable of generating sufficiently large inocula to infect so many individuals. Recreational water centres have been implicated in large community outbreaks of Legionnaires' disease [8,21], but such centres do not exist in the Cerdanyola district, and it was considered highly improbable that the entire infected population

had visited a centre of this type outside the district. Therefore, cooling towers and evaporative condensers were the main focus of the investigation, especially after considering previous experience in Spain [22,23]. In Catalonia, such facilities must be registered, but compliance is not uniform. Visual inspection, as well as aerial inspection using thermal imaging, was employed in the present study. Thermal imaging may be useful to distinguish aerosols emitted from cooling towers, but its usefulness in the summer in a hot climate is debatable.

Since all the cases in the outbreak were diagnosed initially by antigen detection in urine, the search for the source was centred on environmental investigations for *L. pneumophila* sg 1. In such situations, a rapid test, such as PCR, can be useful to determine the degree of *Legionella* contamination of a cooling tower in order to close it or to initiate an immediate hyperchlorination programme. Samples should be collected for the quantitative analysis of *Legionella* and for molecular subtyping. PFGE has limitations if interpreted by the Tenover criteria [24,25], but there is usually 100% identity between the PFGE profiles of clinical and environmental samples in outbreak situations [8,22,23]. Thus, PFGE is a valuable technique when accompanied, as in the present study, by coincident epidemiological data.

The media attention that often follows the first cases of an outbreak can tempt the owners of cooling towers to shut them down immediately or carry out preventive disinfection before inspection by public health authorities. This hinders any investigation, but may help to curtail the outbreak. Although environmental studies may be negative initially, periodic investigation of such facilities can reveal the presence of clones of *Legionella* that are identical to the clinical isolates from cases in the outbreak [23].

The role of the cooling tower in the outbreak described in the present study is evident. Despite the alarm generated among the local population and the progressive increase in the number of cases, the cooling tower continued functioning. The location and size of the tower were precisely what might have been expected in an outbreak with these characteristics. Most patients lived very near the tower, and those living at a distance of >1000 m had a history of having been in the area on different occasions during the 10-day period before the onset of symptoms. Abundant

growth of *L. pneumophila* sg 1 was observed within 48 h of taking samples from the tower, and these were indistinguishable from the clinical isolates from patients, but different from isolates obtained from other sources. Nine days after closing the tower, new cases of pneumonia caused by *L. pneumophila* ceased to appear, which also indicates that this cooling tower was the definitive source of the outbreak.

A limitation of the present study was the absence of a case-control analysis to reinforce the data presented. The absence of alternative sources to those studied, and particularly the difficulty in assessing other possible sources of exposure, given the similar movements of cases and possible controls within such a small area, would have complicated the study considerably. Thus, it is possible that some individuals residing >1000 m from the source were misclassified into the unexposed group. The media attention created by the outbreak, coupled with the concerns of the population of the Cerdanyola district during the outbreak, mitigated against performing a case-control study. It was considered that such a study would not have provided additional evidence concerning the origin of the outbreak and, given the human and economic resources involved, could have delayed the investigation.

Finally, some epidemiological data deserve mention. Within the exposed population, as in other outbreaks, a predominance of males and a high level of smoking were characteristic. Interestingly, when news of the outbreak became public, subtle differences in some of the demographical variables studied were observed. Before the declaration of the outbreak, the cases were older and there was a higher percentage of females. After the declaration, it was noticeable that individuals attended hospital more rapidly following the onset of symptoms, and that the rate of hospitalisation decreased. In contrast, a previous study [9] reported that the age of patients increased after the declaration of an outbreak. This may perhaps be explained by different patterns of accessibility to healthcare services. The case fatality rate in the present outbreak was very low, probably because of its early detection, the availability of the *Legionella* antigen test for urine, and the rapid initiation of effective treatment.

Outbreaks of Legionnaires' disease will continue to occur in the future, but it should be a

public health objective to reduce their number and size [26]. The speed of response determines the dimensions of the outbreak. The systematic use of antigen detection in urine in healthcare centres, compulsory culturing of respiratory samples for *Legionella* when there is a positive test, urgent reporting to health authorities, the investigation of potential environmental sources in the area of infection, the use of a rapid environmental test for *Legionella* to allow immediate decision-taking, and systematic molecular subtyping of all environmental and clinical isolates will contribute to the achievement of these objectives.

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